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Distinct lithium-induced gene expression effects in lymphoblastoid cell lines from patients with bipolar disorder

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Abstract

Lithium is the most commonly prescribed medication for the treatment of bipolar disorder (BD), yet the mechanisms underlying its beneficial effects are still unclear. We aimed to compare the effects of lithium treatment in lymphoblastoid cell lines (LCLs) from BD patients and controls. LCLs were generated from sixty-two BD patients (based on DSM-IV) and seventeen healthy controls matched for age, sex, and ethnicity. Patients were recruited from outpatient clinics from February 2012 to October 2014. LCLs were treated with 1 mM lithium for 7 days followed by microarray gene expression assay and validation by real-time quantitative PCR. Baseline differences between groups, as well as differences between vehicle- and lithium-treated cells

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Conflict of Interest

All authors declare that they have no conflicts of interest.

Contributors

GRF, GDC, CW, and CLB designed the study and wrote the protocol. GRF, GDC and NM performed the cellular experiments. NM performed the qPCR experiments. JZ and ZZ performed the statistical and bioinformatics analyses. JGA, CLB and CQ enrolled subjects in the study. GRF and CW wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

within each group were analyzed. The biological significance of differentially expressed genes was examined by pathway enrichment analysis. No significant differences in baseline gene expression (adjusted p-value < 0.05) were detected between groups. Lithium treatment of LCLs from controls did not lead to any significant differences. However, lithium altered the expression of 236 genes in LCLs from patients; those genes were enriched for signaling pathways related to apoptosis. Among those genes, the alterations in the expression of PIK3CG, SERP1 and UPP1 were validated by real-time PCR. A significant correlation was also found between circadian functioning and CEBPG and FGF2 expression levels. In summary, our results suggest that lithium treatment induces expression changes in genes associated with the apoptosis pathway in BD LCLs. The more pronounced effects of lithium in patients compared to controls suggest a disease-specific effect of this drug.

Keywords

bipolar disorder; lithium; lymphoblastoid cell lines; gene expression; apoptosis; morningness

1. Introduction

Bipolar disorder (BD) is a chronic and severe psychiatric disorder with a prevalence of approximately 1% in global population (Grande et al., 2016). It is a lifelong episodic illness with a progressive course characterized by the occurrence of at least one manic episode (BD I) or one hypomanic and one major depressive episode (BD II). Specifically, manic or hypomanic episodes are states of elevated mood and increased motor drive that impair social or occupational functioning, and a comprehensive biological, social, and psychological approach is required for its treatment and investigation (Grande et al., 2016). Biological rhythms and circadian functioning are also tightly linked to the pathophysiology of BD and have been shown to be significantly impaired in patients (Murray and Harvey, 2010). So far, no biomarker has yet been approved for BD's diagnosis, prognosis, or treatment response (Carvalho et al., 2016; Frey et al., 2013).

Mood stabilizers, including lithium, constitute the most common category of medications used in the management of BD. The mood stabilizing properties of lithium were first discovered in 1949. Since then, it has been considered the first line of treatment for BD (Pisanu et al., 2016b). High variability is generally observed in patient response to treatment, which has led to several investigations of lithium's mechanisms of action and the biological underpinnings of this response heterogeneity. Accordingly, the responsiveness to lithium has been particularly investigated in genomic studies and point to the existence of a distinct, more homogenous phenotype within BD patients (Alda et al., 2005; Grof et al., 2002; Scott et al., 2017) that appears to be stable across life (Berghofer et al., 2013). In addition, as seen in other diseases (Brunner-La Rocca et al., 2001; Potze et al., 2014), lithium might induce different effects in patients compared to controls, and understanding these diagnosis-specific effects might shed light into the genetic make-up of patients and its interaction with lithium.

Different models have been developed for the assessment of the effects of medications in vitro and in vivo. Among cellular models, lymphoblastoid cell lines (LCLs) derived from the

transformation of patients' lymphocytes represent a valid and useful experimental tool (Breen et al., 2016; Hunsberger et al., 2015; Kittel-Schneider et al., 2015; Sugawara et al., 2010). Accordingly, alterations in the expression of specific genes in LCLs might help understand which genes might be linked to lithium treatment, thus shedding light into its mechanisms of action and clarifying the beneficial effects of lithium in patients. With this scenario, in this study we aimed to investigate the effects of lithium treatment on genome-wide gene expression in LCLs from BD I patients and healthy controls.

2. Experimental procedures

Subjects

Sixty-two patients with diagnosis of BD I and 17 healthy controls were recruited at The University of Texas Health Science Center at San Antonio. All patients were recruited from outpatient clinics from February 2012 to October 2014 and diagnosed according to the DSM-IV criteria. Healthy controls were recruited based on the absence of any current or past diagnosis of psychiatric disorders using the M.I.N.I version 6.0 (Sheehan et al., 1997). Written informed consent was obtained from each subject after a complete description of the study, which was approved by the local ethics committee. Mood symptoms were assessed by the 15-item Bipolar Inventory of Symptoms Scale (BISS-15) (Bowden et al., 2007). Subjects were also administered the 13-item Composite Scale of Morningness (CSM) (Smith et al., 1989) for the assessment of circadian functioning, in which scores can range from 13 (extreme eveningness) to 55 (extreme morningness).

Cell culture

Peripheral blood was collected from each fasting subject and LCLs were generated from leukocytes using LeucoPREP brand cell separation tubes (Becton Dickinson Labware, Bedford, MA, USA), followed by their transformation using Epstein–Barr virus (EBV) under standard procedures. After reaching the confluence state, each cell line was adequately prepared for storage in liquid nitrogen. For the actual experiment, cells were thawed and regrown in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 15% fetal bovine serum (Gibco, Carlsbad, CA, USA) and 1% penicillin/streptomycin (Gibco). After 72 hours of stabilization in culture, LCLs were counted and plated in 6-well plates at a confluence of 0.5×10^6 cells/well and treated with medium containing either 1 mM lithium acetoacetate (Sigma-Aldrich) or vehicle (medium) for 7 days (same number of cells/well for all subjects from both groups). The 7-day treatment scheme with 1mM lithium is suggested to mimic a chronic treatment with lithium in patients and has been consistently used in previous studies (Breen et al., 2016; Cruceanu et al., 2012; Emamghoreishi et al., 2015b; Sun et al., 2004). Cells were cultured at 37°C in a humidified incubator with 5% CO₂ for 7 days, and medium was changed after 96 hours in culture. After 1 week of treatment, in which continuous presence of 1 mM lithium was warranted, cells were harvested for RNA isolation.

Cell viability

Cell viability was assessed by the quantification of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction to a blue formazan product by cellular

dehydrogenases, as previously described (Mosmann, 1983), with minor modifications. LCLs were seeded in 24-well plates at a density of 0.125×10^6 cells/well, followed by treatment with 0.1, 1 or 10 mM lithium, as described above. After 7 days of treatment, 0.5 mg/mL of MTT was added to each well. After incubation for 2 hours at 37°C, the cells were centrifuged and pellets of formazan crystals were dissolved with 200 µL of DMSO. The optical density was quantified at 560–630 nm by a microplate reader (Synergy, Biotek). Cell viability is presented as a percentage ratio of absorbance of exposed cells to vehicle cells.

Genome-wide expression

Cells from each subject were collected separately and individually processed for RNA isolation and genome-wide expression experiments, after which the average expression levels for each gene within each group were calculated. Total RNA was isolated from cultured cells using the RNeasy Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. After quantification on NanoDrop (Thermo, Waltham, MA, USA), the integrity of RNA samples was assessed by the Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA) on a Bioanalyzer 2100 (Agilent Technologies), and samples (RNA integrity number >9.8) were subsequently labelled into biotinylated cRNAs with the TargetAmp™-Nano Labeling Kit (Epicentre, Madison, WI, USA). Genome-wide expression levels were measured using the Human HT-12 v4 Expression BeadChip Array (Illumina, San Diego, CA, USA) according to the manufacturer's instructions and scanned on an iScan Microarray Scanner (Illumina). Gene expression data were quantile normalized and exported into text files using GenomeStudio software v2011.1 (Illumina), and data analyses were performed using the R package Bioconductor. Gene set enrichment analysis was performed by the Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 using Gene Ontology (GO) terms. The enriched GO terms were selected based on a false discovery rate (FDR) cutoff < 0.05.

Real-time quantitative PCR

We validated the microarray data by performing real-time quantitative PCR for three differentially expressed genes selected from the previous analysis (PIK3CG, SERP1, and UPP1). Briefly, 500 ng of RNA were reverse-transcribed to cDNA using the High Capacity cDNA Reverse Transcription kit (Life Technologies, Carlsbad, CA, USA). Quantitative PCR was performed in 12 µL-reactions that included TaqMan® Gene Expression Master Mix (Life Technologies) and a TaqMan® Gene Expression Assay (Life Technologies) containing primers and a probe specific for the gene of interest (PIK3CG – Hs00277090_m1; SERP1 – Hs00205386_m1; UPP1 – Hs01066247_m1). Multiplex reactions were amplified and analyzed in triplicates in the QuantStudio® 7 Flex Real-Time PCR System (Life Technologies) using HPRT as the endogenous control (Hs02800695_m1). Relative quantification was analyzed by the delta delta Ct method (Livak and Schmittgen, 2001).

Statistical analysis

Statistical analyses were performed using GenomeStudio (Illumina), R package, and IBM SPSS Statistics 24 (SPSS Inc., Chicago, IL). Independent t-test was used to compare age, chi-square tests were used to compare sex and ethnicity, and Mann-Whitney tests were used

to compare CSM and BISS-15 scores between groups. P-values < 0.05 were considered significant for the demographic data analyses. Gene expression data distribution was tested by the Shapiro-Wilk test and histogram visualization. Differential expression (for both the microarray data and qPCR validation) was assessed at baseline (patients vs. controls) with Mann-Whitney tests and after lithium treatment using Wilcoxon signed-rank tests. Age and sex adjustment was performed using linear model to assess any potential impact of these variables in the prediction of gene expression measures. Correction for multiple testing was performed using the Bonferroni-Holm method (Holm, 1979). Only adjusted p-values < 0.05 were considered significant.

3. Results

Description of samples

Demographic data from the participants are presented in Table 1. Patients and controls did not differ for any of the demographic variables assessed (age, sex and ethnicity), but patients showed significantly higher BISS-15 total scores compared to controls ($p < 0.001$). Moreover, patients and controls showed no differences in the total CSM scores ($p = 0.095$). We also compared the frequency of chronotypes (evening, intermediate or morning type) based on the criteria proposed by Smith et al. (Smith et al., 1989), but no differences were found between groups ($\chi^2 = 0.286$, $p = 0.867$). Sixty-four percent of patients were on medications (Table 1), which included lithium (20.9%), valproate (19.3%), carbamazepine (4.8%), lamotrigine (20.9%), antidepressants (16.1%), quetiapine (25.8%), other atypical antipsychotics (1.6%), and others (37%).

Genome-wide expression analysis

Gene expression was compared at baseline (patients vs. controls) and after lithium treatment (lithium vs. vehicle). No gene was found to be differentially expressed between patients and controls at baseline (vehicle-treated cells) after correcting for multiple testing (adjusted p-values > 0.05). After one week of treatment, 1 mM lithium did not significantly alter gene expression in cells from controls (adjusted p-values > 0.05). However, 236 genes were altered in cells from patients after lithium treatment (160 were up-regulated and 76 down-regulated; adjusted p-value < 0.05 , Table S1). Of note, we confirmed that lithium treatment did not induce changes in cell viability in patients or controls (Figure 1a–c).

Pathway analysis

Pathway analysis was performed considering all differentially expressed genes listed in table S1. Differentially expressed genes in patients were enriched for the terms ‘regulation of programmed cell death’ (GO: 0043067, FDR = 0.020) and ‘regulation of cell death’ (GO: 0010941, FDR = 0.021), suggesting a modulatory role for lithium in the apoptosis pathway specifically in patients (Table 2 and Figure 2). Both apoptosis-related GO terms were enriched with 26 genes that vary in their mechanisms of action in the cell (Table S2). Other pathways not meeting the FDR cut-off criteria are also related to apoptosis and cell death (Table 2).

Validation of microarray data by real-time qPCR

We validated the results from the microarray by assessing the mRNA levels of three genes shown to be differentially expressed after lithium treatment in the initial genome-wide analysis. We selected the top-differentially expressed gene in the cells from patients (UPP1), one differentially expressed gene in cells from patients that was also nominally differentially expressed after lithium treatment in controls (SERP1, nominal p-value = 1.53E-05, FDR-adjusted p-value = 0.528), and one differentially expressed gene that belongs to the apoptosis pathway identified in the GO analysis (PIK3CG, Table 2). As shown in Figure 3, the differences found in the microarray were similarly replicated by the qPCR, with a positive correlation between data from both analyses (SERP1: correlation $r_s = 0.512$, $p < 0.001$; UPP1: $r_s = 0.910$, $p < 0.001$; PIK3CG: $r_s = 0.747$, $p < 0.001$).

Correlations with clinical data

To explore the potential clinical implications of our genome-wide expression findings, we correlated the expression levels of the differentially expressed genes assigned to the apoptotic pathways with the clinical data available for the participants. We found that total CSM scores significantly correlated with the baseline (vehicle) levels of CEBPG ($r_s = 0.235$, $p = 0.027$) and FGF2 ($r_s = 0.210$, $p = 0.049$), while total BISS-15 scores did not correlate with any of the genes. Moreover, the age at onset of illness within patients was significantly correlated with the expression levels of FGF2 after lithium treatment ($r_s = -0.395$, $p = 0.017$), and so did ID3 ($r_s = 0.370$, $p = 0.026$), P2RX7 ($r_s = -0.462$, $p = 0.005$), and TNFRSF10B ($r_s = -0.384$, $p = 0.021$). Only the baseline (vehicle treatment) level of P2RX7 was significantly correlated with the age at onset ($r_s = -0.347$, $p = 0.0036$).

4. Discussion

This study aimed to investigate gene expression alterations underlying the mechanisms of action of lithium in LCLs from patients with BD and controls. Our results suggest that lithium treatment induces changes in expression of apoptosis-related genes, specifically in LCLs from patients with BD. In particular, the more pronounced effects observed in cells from patients compared to controls suggested a disease-specific genotype- and/or phenotype-dependent effect of lithium, which now warrants further investigation.

Apoptotic mechanisms have been consistently shown to be involved in the pathophysiology of BD, with reports in postmortem tissues (Gigante et al., 2011), periphery (Fries et al., 2014; Moutsatsou et al., 2014; Scaini et al., 2017), and other cell types (Schroeder et al., 2016), suggesting a higher proportion of apoptotic cell death and a pro-apoptotic protein profile in patients (Benes et al., 2006; Kim et al., 2010; Politi et al., 2008; Wang et al., 2016). In addition, the role of lithium in the modulation of cell death and apoptosis has been previously suggested by several studies (Bielecka and Obuchowicz, 2008; Breen et al., 2016; Chiu and Chuang, 2010), although the role of specific genes in this mechanism still needs to be unraveled. Top signaling pathways that were suggested to underlie the anti-apoptotic properties of lithium include the modulation of the Wnt pathway (Hu et al., 2011), mitochondrial function and calcium homeostasis (Alda, 2015; Scola et al., 2014), the expression of anti-apoptotic proteins (Dwivedi and Zhang, 2014; Lowthert et al., 2012),

neurotrophic factors (Dwivedi and Zhang, 2014; Emamghoreishi et al., 2015a; Hellweg et al., 2002), among others (Alda, 2015; Bielecka and Obuchowicz, 2008). Accordingly, a recent study has shown that lithium upregulates a gene module specific to apoptosis signaling and exert regulatory effects on apoptosis-controlling proteins involved in mitochondrial and endoplasmic reticulum function in LCLs from BD patients (Breen et al., 2016), which corroborates our findings.

Specifically, lithium modulated the expression of 26 genes involved in the apoptosis signaling pathway in our analysis (7 down-regulated and 19 up-regulated, Table S2), some of which had been associated to BD in previous studies. For instance, a genetic variation at the AKAP13 gene, whose levels were increased by lithium in our experiment, has been previously associated with the efficacy of acute haloperidol treatment (Drago et al., 2013). Moreover, a positive correlation between AKT1 gene expression after lithium treatment and an improvement of depressive symptoms has been reported in BD patients (Machado-Vieira et al., 2015). Of note, previous findings have also linked BD with genetic variants of the AKT1 gene (Karege et al., 2012), and AKT1 expression levels have been shown to be increased in BD patients compared to controls (Gouvea et al., 2016). Interestingly, our results show that lithium decreased AKT1 expression in cells from patients, suggesting the possible effect of lithium on this gene expression. In addition, we observed an increase in the expression of DDIT3 in lithium treatment. DDIT3 is a key modulator of the unfolded protein response activated by the endoplasmic reticulum (ER) stress. This finding was also supported by the literature, with several studies suggesting a key role of ER stress in BD (Hayashi et al., 2009; Pfaffenseller et al., 2014; So et al., 2007), including the effects of lithium on LCLs (Breen et al., 2016).

Our results also showed that lithium altered the expression of FGF-2 in LCLs from patients. FGF-2, fibroblast growth factor 2, is considered to play an important role in the regulation of cell survival. Interestingly, previous reports suggest that patients with BD show reduced FGF-2 mRNA levels in the hippocampus (Gaughran et al., 2006) and increased FGF-2 protein serum levels compared to controls (Liu et al., 2014). The latter finding was suggested to take place as a compensatory neuroprotective mechanism (Liu et al., 2014), which is supported by our finding that lithium increases FGF-2 gene expression in LCLs. Finally, our results also show that lithium increases the expression of P2RX4 and P2RX7, both of which belong to the family of purinergic receptors previously linked to BD pathophysiology (Backlund et al., 2011; Halmai et al., 2013; Mantere et al., 2012).

Importantly, lithium induced a much stronger effect in the cells from patients compared to controls. These results may suggest that there might be some intrinsic genetic factors in BD patients that after lithium treatment alter the expression of genes more markedly, or alternatively, that lithium treatment might be more effective in patients than in controls. In light of this finding, we hypothesized that lithium might be attempting to 'correct' a dysfunctional gene expression network more prevalently seen in patients. The problem with this hypothesis lies on the fact that no significant differences on gene expression were detected between patients and controls at baseline (vehicle treatment). Nonetheless, it might be the case that the dysfunctional gene expression signatures that are altered by lithium are only slightly different between patients and controls at baseline, which would explain the

lack of significance with our relatively small sample size (especially in the control group) and the stringent genome-wide statistical analysis used in this study. In support of this, previous studies have reported on gene expression baseline differences in LCLs from patients and controls (Kittel-Schneider et al., 2015; Squassina et al., 2013), which might also suggest differences in terms of patients' sampling and population between our study and others. An alternative explanation may be that the effects of lithium are highly dependent on the 'diseased' genotype/phenotype. This goes along with the increasing body of evidence suggesting a key role of pharmacogenetics in the response to lithium (Pisanu et al., 2016a). Accordingly, different, more homogeneous phenotypes have been identified within patients regarding their likelihood of showing an adequate response to lithium treatment (responders vs. nonresponders) (Geoffroy et al., 2017; Oedegaard et al., 2016; Scott et al., 2017; Sportiche et al., 2016), and the genetic basis of lithium responsiveness has been consistently shown by several studies (Alda, 2015; Alda et al., 2005; Grof et al., 2009; Grof et al., 2002; Hou et al., 2016). This is also supported by in vitro studies performed with LCLs, in which lithium has been shown to exert specific effects based on the donor's treatment responsiveness (Hunsberger et al., 2015; Milanesi et al., 2015; Squassina et al., 2013). In fact, cellular features of induced pluripotent stem cell-derived neurons derived from B-lymphocytes and fibroblasts have also been recently shown to discriminate between lithium responders and non-responders (Mertens et al., 2015; Stern et al., 2017), suggesting that a further investigation of our findings in regards to lithium responsiveness is now warranted. As an exploratory analysis, we also correlated the levels of the differentially expressed genes enriched for the apoptosis pathway with a couple of relevant clinical parameters available for these samples. As expected, no correlations were found between symptomology (as assessed by the BISS-15 scale) and our gene expression data, which can be explained by the fact that the lymphocytes' transformation into lymphoblastoid cell lines is thought to 'reset' (or at least significantly reduce) the immediate environmental effects seen in the original primary cells (such as the effects of medications and, as suggested by our results, the acute symptoms). Nevertheless, we found significant correlations between morningness/eveningness (assessed by the CSM) and the baseline expression levels of CEBPG and FGF-2. Accordingly, protein members of the fibroblast growth factor family have been previously suggested to present a circadian rhythm in plasma (Haus et al., 2001; Yu et al., 2011). It is likely that the degree to which a patient presents morningness or eveningness is not so much dependent on the environment, but rather on genetic information that remains after the lymphoblasts' transformation. In other words, whereas symptomatology is clearly a 'state' feature (therefore not showing any correlations with measures obtained in LCLs), circadian functioning may be a transmittable 'trait' feature mostly determined by the genotype (Pagani et al., 2016; Zhang et al., 2011). The same can be applied to the age at onset, which also showed sparse correlations with gene expression and is thought to represent a more genotype-dependent characteristic. Accordingly, patients with an earlier age at onset of illness have been shown to present a distinct phenotype of illness with more severe clinical features (Suominen et al., 2007), and may therefore represent a distinct genetically-driven subtype of BD.

Our results need to be interpreted with caution due to some limitations. First, the sample size for the control group is small, which might have led to an underpowered analysis of the

genome-wide lithium-induced effects in their cells. Accordingly, we cannot rule out that at least part of the non-differential expression in controls might be due to the small sample size and not the suggested phenotype-specific effect discussed above. Second, we do not have information on other very important demographic information known to modulate gene expression in patients and controls, such as body mass index or smoking status. Clinically, as discussed earlier, an analysis accounting for the status of lithium responsiveness in patients is warranted and will likely better inform the mechanisms by which lithium induces the resolution of symptoms in patients with BD. Previous studies using LCLs from lithium responders and non-responders suggest specific effects of lithium based on this clinical phenotype. In this sense, an integrated analysis of lithium responsiveness including genotyping information from the subjects may also be desirable given the growing body of evidence suggesting an important role of genetic variants in the clinical response to lithium. Finally, even though LCLs are well established in pharmacogenomic studies, they are also limited when considering the possible alterations found between the cell lines and the primary cells induced by the viral transformation protocol (Breen et al., 2016).

In conclusion, this study suggests that the in vitro lithium treatment of LCLs induces changes in the expression of genes associated with the apoptosis pathway specifically in patients. The more pronounced effects of lithium in patients compared to controls suggest a disease-specific effect of this drug and lay the foundation for future investigations in the pharmacogenetics of lithium and BD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Cell viability after treatment of lymphoblastoid cells with lithium. Lithium at three different concentrations (0.1, 1 or 10 mM) did not alter cell viability assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. Each dashed line represents the 100% viability of vehicle-treated cells. Each bar represents average of the percentage of vehicle $\pm$ standard error of the mean.

Path Designer Genes apoptosis pathway

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Figure 2.

Lithium-induced differentially expressed genes enriched for 'regulation of programmed cell death' (GO ID: 0043067). Only the sixteen genes (out of 26) that show direct or indirect relationships (according to the IPA Knowledge Base) are depicted. Green represents down-regulation of the gene, while red represents up-regulation. The network was made with the software Ingenuity® Pathway Analysis, version 31813283 (IPA, QIAGEN).

Figure 3.

Validation of microarray expression data. A–C) Real-time quantitative PCR validation for the differences in the expression of PIK3CG, SERP1 and UPP1 after lithium (Li) treatment in lymphoblastoid cells from patients with bipolar disorder (BD I). RQ stands for the ‘relative quantification’ calculated by the delta delta Ct method. D–F) Microarray results showing the differences in PIK3CG, SERP1 and UPP1 expression after lithium treatment.

Table 1

Sample demographics

	Controls (n = 17)	Patients (n = 62)	P
Age ^a	37.35 (11.2)	42.6 (11.9)	.108 ^c
Sex (F/M)	7/10	38/24	.138 ^d
Ethnicity (H/N)	6/11	11/50	.127 ^d
CSM (total) ^b	39.0 (9)	35.0 (10)	.095 ^e
BISS-15 (total) ^b	0.0 (1)	6.0 (13)	< .001 ^e
Medication use (%)			
Lithium	-	13 (20.9%)	
Valproate	-	12 (19.3%)	
Carbamazepine	-	3 (4.8%)	
Lamotrigine	-	13 (20.9%)	
Antidepressants	-	10 (16.1%)	
Atypical antipsychotics	-	1 (1.6%)	
Quetiapine	-	16 (25.8%)	
Other	-	23 (37.0%)	

BISS-15 – Bipolar Inventory of Symptoms Scale; CSM – Composite Scale for Morningness; F – female; H – Hispanic or latino; M – male; N – Non-hispanic or latino.

^a mean(SD);

^b median (interquartile range);

^c independent t-test;

^d chi-square test;

^e Mann-Whitney test.

Table 2

Top-ranked enriched GO terms for differentially expressed genes after lithium treatment

Rank	Term	Genes	P-value	FDR
#1	GO:0043067 - regulation of programmed cell death	26	1.21E-05	0.020181*
#2	GO:0010941 - regulation of cell death	26	1.29E-05	0.021493*
#3	GO:0042981 - regulation of apoptosis	25	3.05E-05	0.050907
#4	GO:0010033 - response to organic substance	23	4.88E-05	0.081485
#5	GO:0043065 - positive regulation of apoptosis	17	5.86E-05	0.097767
#6	GO:0043068 - positive regulation of programmed cell death	17	6.36E-05	0.106145
#7	GO:0010942 - positive regulation of cell death	17	6.72E-05	0.112076
#8	GO:0033554 - cellular response to stress	19	1.50E-04	0.249537
#9	GO:0006974 - response to DNA damage stimulus	15	1.58E-04	0.26307